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Further Notes on the Pigmentary Behavior of *Chaetodipterus* in Reference to Background and Water Transparency

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(Plates I & II)

It has been shown by Breder (1946) that very small individuals of *Chaetodipterus faber* (Broussonet) appear in a coal black stage under certain conditions. When viewed against a light sand background on which similar-sized black gastropods and black infertile pods of the red mangrove were scattered, the small fish effectively vanished from sight. This black color phase was seen in fishes which were of about 10 mm. in length and which always inclined to one side. At larger sizes they were found to show the characteristic black and white vertical bars and upright position. The above observations were made on the west coast of Florida.

Subsequently, observations differing from these were made in the Bahamas, at the Lerner Marine Laboratory (Breder, 1948 and 1949). Larger-sized fishes, up to and including those a foot or more in length, were found there to appear not infrequently in a similar black phase. At such times they always lay on one side on the bright, light colored sand, appearing much like a piece of drifting trash, and could be easily overlooked. In Breder's 1949 paper it was suggested that the difference between the behavior of these fishes at the two places might be associated with the difference in the transparency of the water, that on the Florida west coast being notably turbid while the Bahamas water is remarkably transparent.

Various experiments were undertaken with some of these Bahaman fish in an effort to determine more clearly the basis of the differential behavior. In aquaria, under the most diverse conditions, they were found to swim upright and show at least some traces of the vertical bars, although the bars were generally less distinct when a minimum of dark objects was seen by the fish. This is, of course, in keeping with what had already been found.

Some of the experiments included presenting the fishes with variously painted backgrounds, such as broad dark vertical bars. In one series of experiments, quite accurate drawings of *Chaetodipterus* in groups in the solid black phase and in the barred phase were exhibited to a similar-sized test fish. None of these tests was able to make the fishes, solitary or in groups, obliterate the vertical bars, or recline as do the dark ones in the sea against a light sand background, or show especially vivid bars. It is to be noted that this is one of the species which does not show the classical concentration of melanophore granules in response to adrenalin (Breder & Rasquin, 1950).

A favorable situation led to the following clarification of some of the details of the characteristics which an environment must have to elicit the full expression of this dark coloration and reclining posture. A single individual about 3 inches long, in the dark phase, found reclining on its side near the laboratory dock, was transferred to a shallow circular concrete pool 12 feet in diameter. Here, over a bottom of clean light sand, the fish continued to perform as it had in the sea. Plate I, Figure 1, shows its appearance at this time. Both in this pool and earlier in the sea it permitted a very close approach and sometimes netting by the observer, simply lying very quiescent, but in about half the approaches it would dart away a short distance when very closely and persistently pursued. In this pool it normally took a position somewhere near the center and huddled toward the light blue walls of the construction.

After being transferred to a small aquarium, 2 × 1 × 1 feet, with a similar white sand bottom, the fish retained both its position and coloration as is shown in Plate I, Figure 2. These it held even when presented with various targets of black stripes and the figures of banded fishes which had been used earlier in the tests on other fishes. It did, however, show light bands but not very strongly, seemingly as a "fright" reaction in response to such things as taps on the glass walls of the tank.

On the coming of night and consequent lowering of light intensity the fish always swam erect and showed its bands. Such a condition is shown in the photograph in Plate I, Figure 3, taken by flashlight. Thus this behavior is evidently associated with light intensity, "fright" and a variety of visual stimuli, the latter perhaps being the least potent of the three influences. The puzzling responses studied earlier, wherein the fishes showed bands under all manner of aquarium fittings, were thus evidently actually in response to both fright and lowered light intensity. In these former experiments the aquaria were wrapped with paper on at least three sides and sometimes four, to insure the fish seeing a minimum of distracting objects, thus rather drastically reducing the light intensity.

Inasmuch as light intensity is evidently involved, and the turbidity of the water on the west coast of Florida reduces underwater illumination as compared with the Bahaman situation, the following experiment was undertaken. A suspension was made of pulverized charcoal and this was poured into the aquarium in broad daylight when the fish was in a dark, side-resting condition as shown in Plate I, Figure 2. The suspension made the water turbid and the larger particles settled out, transforming the aquarium bottom from one of nearly white sand to a mostly dead black condition. Immediately the fish erected itself and displayed the light, nearly white vertical bars, as is shown in Plate I, Figure 4, where the fish can be only faintly seen because of the turbid water. The following day the water had cleared but the bottom was largely black and the fish retained its stripes in bright daylight but over this dark bottom. This is shown in Plate I, Figure 5. Light sand was then introduced into the aquarium gently, through a small pipe, in order to bury the layer of charcoal. As this was late in the day, an artificial light was arranged so as to eliminate the effect of the decreasing light intensity. The fish returned to its black coloration and retained it long after daylight had faded. This is shown in Plate I, Figure 6. When this special light was extinguished, the fish then showed its bands as it had on other nights.

The following day the fish showed its daytime black color appropriate to a light sand background. Netting caused bars to reappear. It was then returned to the circular pool where it immediately resumed its dark phase and acted as it had before the aquarium experiments were undertaken. Late this day a small dark piece of Sargassum weed was dropped into the pool and by the following morning it had drifted to the outlet pipe. The fish was found under it and beside the black outlet pipe, vertical and in the strong black and white banded phase. When the fish was chased away from this shelter, it immediately obliterated the bands and reclined as a black object near the center of the pool on the light sand. It is to be especially noted that the outlet and inlet pipes in this pool were vertical and black (hard rubber) but that no attention had been paid to them by the fish until a sheltering, and shadow-casting, object was also associated with them. Similarly, Breder (1948) observed a large black fish, off the laboratory dock, which drifted slowly along the bottom on its side until it approached the pilings. It then became erect and showed its strongest bars as it swam among the rather thin piles supporting the dock. At that time it was thought that it was the sight of the dark vertical lines of the piles which elicited the response. In the light of the present series of experiments it would seem that this behavior is more probably referable to the shadow of the dock and the consequent lower light intensity, than to any definite retinal image.

The bold pattern referred to in this discussion is not to be confused with minor lightenings of the light barred areas which flash faintly in an evanescent manner following all manner of stimuli, including minor "frights" or the sight of food. These lesser responses are evident if watched for closely, and probably have a significance analagous to the twitching of a fin which these, and in fact most fishes, show under similar situations. They seem to be nothing more than nervous "starts." This individual, it must be emphasized, was from the first a most tractable aquarium inmate. An hour after it had been placed in the aquarium the fish acted as though it had always been there. It would investigate a finger outside the glass and it fed freely from this time on. When the light sand was introduced in the course of the experiments, the fish butted and bit at the pipe. It was clearly not nearly so timid as the fishes examined previously, which are referred to earlier in these notes.

The above experiments were performed on this single individual in November, and the fish was maintained in the laboratory until May when it was reintroduced to the circular pool with a light sand background. It responded in a manner strictly comparable to its former behavior and differed only in that the blackening was not quite so complete and the reclined position not so nearly horizontal. This may be associated with the greater age of the fish, as there is considerable evidence that this behavior is most definite in the smaller sizes. On the other hand, it may be more truly associated with the long sojourn in aquaria. It is a commonplace among aquarists that many fishes after long residence in aquaria tend to show less vigor in their various responses than do wild fishes. Such a slackening of behavior vigor may be associated with waning health but certainly, in many cases, it is not so modified. In these instances it would seem to be more a matter of dropping old habits and developing new in accordance with the radical change in environment, brought about by moving from the open sea to a small aquarium, with consequent absence of predators and complete change in the manner in which food is presented or found.

Whether any or all of the above noted matters incident to captivity had anything to do with this slight change in pigmentary behavior is uncertain but they are mentioned here to indicate that the authors have not been unmindful of the possibility of such influences affecting the results. It is believed, moreover, that in the present instance the reduced light to which the fish was exposed in the laboratory aquarium for the period of some months was sufficient to induce a considerable reduction in the numbers of dermal melanophores. It may be noted that *Chaetodipterus* lives well, and for years, in public aquaria but little by little becomes much lighter. This lightening evidently results in part at least from the elimination of melanophores in the comparatively low light intensities of such places.

Because of the "opposite" pigmentary behavior as compared with that of usual background-matching species, the influence of melanophore-affecting hormones and other substances was investigated, as has been noted in passing by Breder & Rasquin (1950). The precise nature of these experiments is given below.

One specimen which weighed 276 grams and measured 192 mm. in standard length was injected with 2.8 cc. adrenalin 1:1,000. Throughout the observation period of four hours the injected fish remained noticeably darker than the uninjected control. Two minutes after injection, the injected fish was darker over the dorsal surface than the control; after six minutes it was still darker than the control but showed small white patches in the light bars. Ten minutes after injection the fish was darker than the control from the mid-dorsum down to approximately the lateral mid-line. It remained definitely gray where the control showed bold white bars. The black bars of the injected fish seemed less definitive than those of the control, although this may have been owing to less contrast of color offered by the injected fish. Two hours after injection the iris of the injected fish was white and the whole animal was quite dark, although not sufficient to eliminate the barred pattern entirely. After three hours the coloration of the iris had returned to normal while a mottled appearance was still evident on the body. After four hours the coloration of the injected fish was nearly back to normal, that is, it was nearly like that of the control. The following morning the injected fish and the control were indistinguishable.

Some observations were made on the pigmentary reaction to different backgrounds of three small individuals in a 15-gallon tank. No adrenalin injections were made. In a tank devoid of any plants or shells, with a bottom of white sand and with clear glass sides, the three fish assumed an all-over dark coloration with the lighter bands showing faintly. With a white sand bottom and with the sides of the tank covered with white paper, the coloration remained very dark with faint lighter bands. The fish appeared somewhat disturbed by this environment; they huddled together and were inclined to lean over to one side (Plate II, Figure 1). They were seen in the same dark color phase at night when the lights were suddenly flashed on in the laboratory.

In a tank with the slate bottom uncovered and the glass sides covered with black paper, the fish became lighter and the bands were more clearly marked (Plate II, Figure 2). They kept this banded condition when surrounded by black but quickly darkened when one paper side was removed for observation. All the fish swam upright in the dark tank and were not seen to lean over to one side as they did in the white tank.

With the sides of the tank covered with two-inch vertical black and white stripes, the fish assumed their bold black and white pattern. This reaction was not a quick one, but took about a half hour to occur. The fish again darkened quickly when one paper side was removed.

Paper images of the fish of approximately the same size were introduced into the experiment. They comprised white images on a black background, black images on a white background and white-barred images on a black background. The pigmentary reactions of the fish were the same when they were surrounded by any of these as backgrounds. They responded with the bold banded phase, even when black images on a white background were used (Plate II, Figure 3).

One of these three fishes never gave as positive reactions as did the other two; it always remained somewhat darker and was seen more often in an inclined position than either of the others. It is possible that this was also an "emotional" reaction of some sort, especially as a dark phase is generally typical of most teleosts in the lowest position in a hierarchy, for this particular fish was annoyed and pecked at by the other two.

This type of pigmentary behavior, of which the present authors are evidently the only ones to take cognizance, at first glance might seem at wide variance with many of the pigmentary studies of recent years which have been broadly summarized by Sumner (1939), Walls (1942), Parker (1948) and Fox (1953). It is believed that the described observations on behavior can be entirely explained on the basis of experiments already performed on other fishes by various investigators. A considerable portion of it can be ascribed to the now well-established fact that tested fishes of various species show pigmentary responses both to light intensity and to the ratio of incident light to that reflected from the background. Walls (1942) treated the matter as follows: "If the fish were responding merely to the amount of light entering the eye, it should give the same responses to a brightly illuminated dark background as to a dimly illuminated white one—which would not adapt the fish at all! Instead, however, the shade assumed by the skin of the fish is always (unless the intensity of the incident light is very low or extremely high) in accordance with the albedo of the substrate—the percentage of incident light which the substrate reflects." Brown (1936) nicely demonstrated that the minnow *Erycimba* clearly responded to both variation in light intensity over a single background and to variations in the ratio of incident to reflected light, by using uniform light intensity and backgrounds of various degrees of reflectivity. Both his series of experiments were carried to the limit of the ability of the melanophores to respond; that is, to disperse or concentrate their melanin granules. Carried beyond, there was no further change. That is to say, below 0.000053 foot candles they showed no further concentration, as such was evidently impossible, while on the same black background, above 1.75 foot candles there was no further dispersion. The dispersion of the melanin was found to be proportional to the log of the foot candles at intermediate light values. Using the minimum light value necessary to produce complete dispersion on a black background, Brown varied the latter through various shades of gray to white; a light gray background which reflected 0.1411 foot candle of the incident 1.75 foot candles (an albedo of 12.4) was sufficient to produce full concentration of the melanin granules. The behavior at the other end of the series could not be tested because of the nature of the experimental arrangement whereby the minimum light intensity necessary to produce complete dispersion on black was employed.

The above-described experiments are sufficient to explain why dark or completely black fishes may show a pattern when the light has been reduced to a point where the presence of such a pattern cannot be seen. This would seem to have nothing to do with whether the fish tends to match the background or to contrast with it, but suffices to explain Figure 3 of Plate I. The phenomenon of a fully black fish on a light ground is apparently confined to places subject to very great light intensities. These often run up to and in excess of 6,000 foot candles in summer and usually well over 2,000 in winter at Bimini. Also the albedo is much smaller in such places than in most other natural environments, reaching values at least as small as 3.00. There is thus much less contrast between background and incident light than in places with darker background and less transparent water. That is, the sensible differential is much reduced, and the retinal polarizing effect is minimized. As noted, at these times the fishes recline so that one eye looks skyward and the other down, rather effectively neutralizing the polarization of the retina as compared with the condition when the fish is in the usual vertical position. Each eye, while seeing a different field, one differing from the other by the difference between the incident light and the reflected light, is nevertheless seeing a comparatively uniform field of very considerable brightness. This in itself may make it impossible for the fish to respond clearly to the albedo, responding instead overwhelmingly to the great light intensity, resulting in its darkening irrespective of the fact that the background is very light. This is the equivalent of saying that the polarizing effect of the visual field is essential in order for a fish *not* to respond only to the light intensity. Evidently only in regions of extremely clear water with an exceedingly light bottom is the described phenomenon possible.

The fact that these fish show their pattern in turbid water would follow from the above as turbidity would increase the value of the albedo because the incident light, whatever its value, only passes from the surface to the eye of the fish, while that of the reflected light has the much greater path; from the surface to the bottom and back to the eye of the fish. Only in perfectly transparent water is the consequent reduction in light negligible. This is because the longer passage of the reflected light in even very slightly turbid water results in much greater filtering. Another type of behavior of *Chaetodipterus* which is encountered at Bimini and other places with similarly clear water, which has not yet been noted, is associated with deep water. In such places schools of five hundred or more individuals of medium to very large size may sometimes be seen, usually resting quietly and all headed into the current. Such schools have not been seen in depths of less than about six fathoms and the fish were always in a strongly barred phase. At such depths the growth-incrusted bottom does not reflect nearly as much light as the sandy shoal waters and consequently the albedo is of appreciable size.

That such fishes as *Chaetodipterus* do not show a general concentration of their melanin granules in response to an injection of adrenalin may be interpreted as follows. After injection of adrenalin the actual dispersion of the melanin granules in the lighter bars, the only place in the body where such activity could be detected, can only mean that the locus of the differential behavior between the two types of fishes is not to be found so much in a differently performing endocrine system but rather in a differently reacting set of target organs. That is to say, the same hormone, adrenalin, induces concentration of melanin granules in the dermal melanophores of *Gambusia,* but its dispersion in equivalent melanophores in *Chaetodipterus.* It is also to be recalled that melanophores of the iris and the meninges in both cases respond by concentrating their granules, as shown by Breder & Rasquin (1950).

In addition to this difference in cellular response it is probable that there is also a more subtle difference in the responsiveness of these pigmentary effects to nervous control. It is obvious that there is here an indication of a lessening endocrine control and an increasing nervous control with the teleosts of the more advanced grades. All the forms known to the authors which show this type of reversal of reaction are acanthopterygians, while the most typical examples of the simple background-matching types are non-acanthopterygians. The fact that these fishes showed faint evidences of momentary lightish bands as a reaction to "fright" when in the black phase, and a darkening of the bands under similar stimulae when in the banded phase, is suggestive of strong nervous control. Such clearly nerve-controlled changes are not evident in the more slowly responding melanophores of most non-acanthopterygians. Actually fishes from more turbid regions, *Chaetodipterus* included, generally show all-over lighter phases. In this extremely clear water, however, these fishes which are so much exposed to strong light have no doubt built up their complement of melanophores to maximum. The concentration of melanin in the areas which remain dark even when the fishes show their light bars is so great that it is doubtful if any hormonal application could cause a visible change in a short time. Evidently a sufficient reduction in the number of melanophores, to permit the noting of hormonally induced changes, could be effected by keeping the fishes under low light intensities for the required time. Whether there is an inverse behavior shown by the guanine present, as Hitchings & Falco (1944) showed for other fishes, cannot be established by these considerations.

A significant side to the differences in chromatic behavior which these fishes show under different conditions of turbidity is the fact that on the west coast of Florida *Chaetodipterus* is known to the natives as "white angel." This name is not used in Bimini, but instead the species is called "chirivita."[1] This appelation, elsewhere used for the very dark colored *Pomacanthus,* has evidently become transferred to *Chaetodipterus* at this place, while *Pomacanthus* is called "black angel."

The intermedin used (Choay) was found to be inactive on the melanophores of many different species of acanthopterygians. It was found to modify the melanophores of the freshwater characin *Astyanax* and the marine cyprinodont *Gambusia* but not to the extent of full granule dispersion. The biological assay for this hormone is performed on the erythrophore system of *Phoxinus laevis* and all fishes on which it was used responded by dispersion of granules in the erythrophores and xanthrophores. Because *Chaetodipterus* did not respond to this preparation of intermedin, it is not to be inferred that the melanophores of this species are not under control of the intermediate lobe of the pituitary, although, as has been noted, the nervous system plays a more dominant role.

The melanophore - dispersing hormone of Armour was not available at the time the above experiments and observations were carried out.

We wish to express our thanks to Miss Carol Mosher for technical assistance in connection with the November experiments and to Mr. William Clarke for observations in May which we were unable to make personally.

[1] Pronounced by the natives as "cherry-wheat-ah," which is in close agreement with their general habits of pronunciation.

Summary

1. The melanophores of the meninges and iris of *Chaetodipterus faber* concentrate their melanin granules on the injection of adrenalin, but those in the dermis simultaneously disperse their granules.

2. This species responds both to changes in light intensity and to the ratio of incident to reflected light from the background or albedo.

3. These conditions are evidently responsible for the fish showing a black and white banded phase in moderate light intensity with a large albedo, as in turbid water against a dark or mottled background, and in very low light intensities.

4. Alternatively they also cause the fishes to show a solid black phase in intense light against a very light background in clear water where the value of the albedo is very small.

5. These conditions lead to the interesting situation under natural conditions of a banded fish becoming inconspicuous against a variety of mottled backgrounds and again inconspicuous against a very light background by becoming uniformly black and appearing as a bit of sea bottom litter.

6. Accompanying these chromatic changes are appropriate attitudes, in the banded phase the fish swimming upright in ordinary fish fashion but in the black phase reclining quietly on the bottom or drifting slowly close to it propelled only by the hyline dorsal and pectoral fins.

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EXPLANATION OF THE PLATES

Plate I. Pattern reactions of a single individual *Chaetodipterus*.

Fig. 1. Behavior in a circular pool with light sand background. Fish black and reclining, as it did in the ocean.

Fig. 2. Identical behavior in a small aquarium floored with light sand.

Fig. 3. Nocturnal pattern, taken by photo-flash.

Fig. 4. Pattern in daytime after water had been turbidified.

Fig. 5. Effect of darkened bottom and clear water.

Fig. 6. Effect of renewed light bottom at night with artificial light. Aquarium photographs by C. Mosher.

Plate II. Pattern reactions of *Chaetodipterus*.

Fig. 1. Darkening of three individuals with the aquarium surrounded by white paper and light sand bottom, with no dark objects in the visual field. In these three photographs the paper on one side of the aquarium was necessarily removed just before the photograph was taken. This did not visibly affect the first two but did lighten the last, which effect increased after photography.

Fig. 2. Pattern of three individuals in an aquarium surrounded by black paper and a black slate bottom. The light appearance of the bottom is a refractive effect.

Fig. 3. Similar pattern shown by a single individual to which are displayed two black paper cut out "fish" with the aquarium surrounded by white paper and with a light sand bottom.